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Protective effect of *Cornus walteri* Wangerin leaf against UVB irradiation induced photoaging in human reconstituted skin

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ABSTRACT

Ethnopharmacological relevance: *Cornus walteri* Wangerin has been used in oriental traditional medicine for the treatment of antidiarrheal and inflammation.
Aim of the study: The efficacy of *Cornus walteri* Wangerin on skin anti-photoaging was investigated.
Materials and methods: Hydrolyzed *Cornus walteri* Wangerin leaf was tested for the anti-photoaging effects against ultraviolet B (UVB)-induced matrix metalloproteinase (MMP)-1, pro-inflammatory cytokines using human reconstituted skin (KeraSkin™-FT) and also tested for elastase activity *in vitro*. The MMP-1 and pro-inflammatory cytokine levels of the extract were evaluated by enzyme-linked immunosorbent assay (ELISA).
Results: The extract of hydrolyzed *Cornus walteri* Wangerin leaf (CWE) had the elastase inhibitory activity (IC_{50}: 0.457 mg/mL). CWE inhibited MMP-1 expression up to 61% in comparison with the control group which was not treated using CWE, but exposed to UVB. CWE also showed an inhibitory effect on releasing pro-inflammatory cytokines (IL-6 and IL-8) in KeraSkin™-FT (30% and 57% inhibition at dose of 50 μg/mL, respectively).
Conclusion: CWE is a promising anti-photoaging agent for the treatment of UVB-induced skin.

1. Introduction

Skin is the largest organ of a human body and constantly exposed to potentially harmful compounds and radiation. Nowadays, human skin is prone to be exposed to ultraviolet (UV) irradiation with the increasing rate of stratospheric ozone depletion. UV light affects human skin in different ways depending on its wavelength. UVA (320–400 nm) effects are primarily oxidative in nature. UVC (200–290 nm) hardly reaches the surface of the earth. UVB (290–320 nm) is mainly absorbed in the epidermis (Krutmann and Schroeder, 2009) and considered to be the causative agent of many issues caused by UV. In particular, UVB irradiation causes skin photoaging. It is well known that chronic exposure of human skin to UVB radiation results in photoaging and induces the production of matrix metalloproteinases (MMPs) and reactive oxygen species (ROS) (Ho et al., 2005; Sesto et al., 2002). Free radicals interact with the transcription factors that bind to the specific sequences of DNA and thus control the transcription of genetic information from DNA to ribonucleic acid (RNA). They regulate transcription factors such as activator protein 1 (AP-1), and a nuclear transcription factor kappa B (NF-κB). AP-1 induces the expression of metalloproteinases that break down existing collagen, resulting in skin wrinkling formation (Farris, 2007; Fisher and Voorhees, 1998). Matrix metalloproteinases (MMPs) are a family of structurally related, zinc-dependent endopeptidases collectively capable of essentially degrading all components of the extracellular matrix (ECM). MMPs play a role in pathological conditions with the excessive degradation of ECM, such as dermal photoaging (Saarialho-Kere et al., 1992). For instance, MMP-1 initiates the cleavage of fibrillar collagen (Type I and III in skin) at a single site within its central triple helix. Once cleaved by MMP-1, collagen is further degraded by the elevated activities of MMP-2 and MMP-9 (Varani et al., 2002). NF-κB stimulates the transcription of pro-inflammatory mediators including interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) (Farris, 2007; Senftleben and Karin, 2002). These pro-inflammatory

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[2] Hyun-Chul Park and Taek Kyu Jung have performed the experiments.
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[5] Mi Jin Kim has provided reagents/materials and helped to revise the manuscript.
[6] Kyung-Sup Yoon has helped with regard to data interpretation and manuscript evaluation.

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mediators help to further activate the transcription factors AP-1 and NF-κB, resulting in damage (Palmer and Kitchin, 2010).

Cornus walteri Wangerin is a deciduous shrub that grows in the valley areas of Asia, especially Korea and China. In China, it has been reported that fruits and leaves of *Cornus walteri* Wangerin have been used for the treatment of skin inflammation or boils caused by lacquer poison in Zhongyaodacidian (encyclopedia of Chinese Materia Medica) (Kim, 1999). In Korean folk medicine, the leaves have been used as an antidiarrheal (Choi et al., 1998; Yook, 1993). The presence of metabolites including gallic acid and flavonoids from previous investigations on *Cornus walteri* Wangerin was confirmed (Choi et al., 1998). *Cornus walteri* Wangerin has various therapeutic properties, including anti-hyperglycemic, anti-obesity effects, and anti-inflammation (Lee et al., 2011; Park and Cha, 2009). Notably, *Cornus walteri* Wangerin extract also possesses antioxidative properties. However, few studies have been carried out *Cornus walteri* Wangerin effects on UVB-irradiated skin. Therefore, the aim of the present study was to examine the ability of *Cornus walteri* Wangerin extract to protect skin against photoaging in human reconstituted skin.

2. Materials and methods

2.1. Preparation of CWE

Cornus walteri Wangerin leaf was purchased from the special herb shop named GUmediherb in South Korea. However, CWE was prepared by the authors following the method described in the previous study (Park et al., 2014). Briefly, *Cornus walteri* Wangerin leaf was extracted twice with 75% aqueous ethanol under reflux at 60–90 °C for 4 h, filtered, and evaporated under reduced pressure. The 75% aqueous ethanol extract was dissolved in 10% aqueous ethanol and then hydrolyzed by Novozyme®33095 (Novozyme, Denmark) at 50–60 °C for 20 h and fractionated by ethyl acetate. The final residues, hydrolyzed *Cornus walteri* Wangerin leaf extract (CWE), were freeze-dried and stored in a closed container until use.

2.2. High-performance liquid chromatography (HPLC) analysis of CWE

HPLC was performed on the Agilent Technologies 1200 series coupled with UV detector, and auto-sampler. The HPLC profiling of the CWE was carried out on C^{18} column (250×4.6 mm, 5.0 μm, Zorbax Eclipes Plus, Agilent, USA). The separation was conducted using a liner gradient from 0.1% formic acid in H_2O to 0.1% formic acid in acetonitrile for 40 min at a flow rate of 0.8 mL/min with detection at UV_{396}. Standard materials were used for (+)-catechin, and quercetin (Sigma, USA).

2.3. Elastase inhibition assay

The elastase activity was evaluated the method previously reported by Kraunsoe et al. (1996) with minor modifications. In order to evaluate the inhibition of the elastase activity, the amount of released p-nitroaniline, which was hydrolyzed from the substrate, N-succinyl-Ala-Ala-Ala-p-nitroanilide, by elastase, was assayed by measuring absorbance at 405 nm. In brief, the reaction was carried out in a 200 mM Tris–HCl buffer (pH 8.0) containing 5 mM N-Suc-(Ala)3-p-nitroanilide and 10 μg/mL elastase. Plant extract was added to the reaction mixture to reach designed concentrations (0.1, 0.2, 0.5, and 1 mg/mL) and elastase inhibition was assessed at 25 °C. The reaction mixture was pre-incubated for 15 min before adding the substrate. Blanks contained all the components except the enzyme. Ursolic acid (UA) was used as a positive control. The change in absorbance was measured at 405 nm using a microplate reader (Bio-Tek, USA). The percent inhibition of elastase was calculated as follows:

$$\text{Inhibition (\%)} = [(A-B)-(C-D)]/(A-B)\times 100.$$

where A is the absorbance of the control with the enzyme, B is the absorbance of the control without the enzyme, C is the absorbance of the test sample with the enzyme, and D is the absorbance of the test sample without the enzyme. All experiments were carried out in triplicate.

2.4. Human reconstituted skin

The 3D human skin model (KeraSkin™-FT) and growth medium were purchased from MCTT Corp. (Korea). This model system consists of normal human epidermal keratinocytes and normal human dermal fibroblasts. The KeraSkin™-FT model exhibits *in vivo*-like morphological and growth characteristics which are uniform and highly reproducible. The KeraSkin™-FT consists of organized basal, spinous, granular, and cornified layer analogous to those found *in vivo*. The KeraSkin™-FT is mitotically and metabolically active produced in cell culture inserts. The KeraSkin™-FT is delivered at 4 °C in a multiwell plate filled with an agarose-nutrient solution in which they are embedded. Growth medium for the KeraSkin™-FT, cooled with gel refrigerant, is delivered along with the KeraSkin™-FT. Upon receipt, the KeraSkin™-FT was equilibrated at 37 °C, 5% CO^2 in media supplied along with the kit for 24 h and maintained. Throughout the experiment, the KeraSkin™-FT was maintained in 6-well culture plates at the air–liquid interface with the lower dermal side of the tissue exposed to media and the upper epidermal stratum corneum exposed to air.

2.5. Cell viability

The cell viability was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Following the treatment with CWE, the growth medium was removed and further incubated with 300 μL of MTT (0.3 mg/mL) for 3 h at 37 °C in a 5% CO^2 incubator. At the end of the incubation, an excess MTT was removed and 2 mL of isopropanol was added to extract the reduced formazan from the tissue, which was incubated for additional 2 h. After formazan extraction, 200 μL extraction solutions were transferred to a 96-well plate, and the absorbance of the extracted purple dye was measured at 540 nm using a microplate reader (Bio-Tek, USA).

2.6. Treatment and UVB irradiation of KeraSkin™-FT

For UVB irradiation, the growth medium for KeraSkin™-FT was replaced with PBS, and the KeraSkin™-FT was exposed to UVB (150 mJ/cm^2). After the UVB exposure, the culture inserts containing the skin samples were placed in fresh media and then the KeraSkin™-FT was treated with or without CWE. After 12 h of incubation, the KeraSkin™-FT was used for MTT assay, and growth medium was collected and stored at −80 °C for future analysis by enzyme-linked immunosorbent assay (ELISA).

2.7. Analysis of cytokines by using ELISA

Following the UVB irradiation, KeraSkin™-FT was incubated with the presence or absence of the CWE at designed concentrations (0, 1, 20, and 50 μg/mL) for 12 h. After the treatment, growth medium was collected to analyze the secretion of cytokines (IL-6, IL-8, and TNF-α) using ELISA kit (R & D Systems Inc., USA). The individual steps were performed according to the manufacturer's instructions. All samples were tested in triplicate.

2.8. Analysis of MMP-1 by ELISA

The secretion of total MMP-1 in the growth medium of KeraSkin™-FT was measured by standard sandwich ELISA (Amersham, UK). The ELISA was performed following the manufacturer's instructions. All